

EXHIBIT B

Materials and Methods

Cell culture and transfection

The ovarian cancer cell line NIH-OVCAR-3 (for study on DNMT3B, C11orf17, CHEK2, FGFR1, KITLG genes) was grown in RPMI-1640 medium (WISENT, St-Bruno, Quebec, Canada) supplemented with 20 % fetal bovine serum (FBS) and 0.01 mg/ml of bovine insulin. TOV-112D cells (for study on BMP4 gene) were grown in OSE medium (WISENT) and supplemented with 10 % FBS. All medium contain L-Glutamine (2 mM) and were incubated at 37°C and 5 % CO₂. Cells were seeded in 6-well plates (350 000 cells/well) and were immediately transfected using 3 ul/well of Lipofectamine 2000 (Invitrogen) as recommended by the manufacturer with 2'OMe antisense (400 nM): TOSS* (an oligonucleotide that is complementary to a specific region upstream of a splice site and having a tail for binding a protein moiety - 400 nM), As* (a classical antisense oligonucleotide - 400nM), A1Casp8-4 (a negative control - 400nM) or TOSS Allstar (a negative control - 400nM). All oligos were purchased from IDT and are made exclusively with 2'OMe RNA (see Table 1 of Exhibit C for a description of the oligos).

Total cell RNA extraction

Twenty-four hours post-transfection, single-step total RNA extractions on human ovarian cancer cells were done with TRIZOL reagent (Invitrogen, Burlington, ON, Canada) as recommended by the manufacturer. The RNA concentration and purity (260/280) was measured using NanoDrop ND-8000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). All RNA samples used showed a 260/280 ratio higher than 1.8. RNA integrity assessment using the Agilent 2100 bioanalyzer (Agilent technologies, Santa Clara, CA). Samples with a RIN (RNA Integrity Number) higher than 9 were used.

Reverse transcription (RT) and real-time polymerase chain reaction (PCR)

- a. TaqMan qPCR approach (for DNMT3B, C11orf17, BMP4, CHEK2, FGFR1 genes)

Following these steps, each RNA sample (2 ug) was subjected to a reverse transcriptase reaction using Omniscript RT kit (Qiagen) following the manufacturer's protocol. For each sample, two distinct RT reactions were done, one specific for each isoform (long and short), using specific reverse primer (refer to Table 2 of Exhibit C). Quantitative Real Time PCR was done by using 7500 RealTime PCR system (Applied Biosystem) and TaqMan technology. For each specific event to quantify, we have designed one specific TaqMan and a pair of primers (forward and reverse) (see Table 2 of Exhibit C) that